

Quantitative determination of Direct LDL Cholesterol in serum/plasma
Only for In Vitro Diagnostic use

ORDER INFORMATION

REF	Cont.
DHDL 20	1 X 20 ML
DHDL 40	1 X 40 ML
DHDL 80	2 X 40 ML

CLINICAL SIGNIFICANCE

Lipoproteins serve to solubilize and transport cholesterol and other lipids in the bloodstream. Different lipoprotein classes have been shown to have various effects on the progression of coronary heart disease (CHD). Low density lipoproteins are associated with increased risk and are seen as a key factor in the pathogenesis of atherosclerosis and coronary artery disease.

The measurement of LDL cholesterol (LDL-C) is a powerful predictor of CHD that provides the opportunity for early diagnosis and intervention to halt the progress of cardiovascular disease

PRINCIPLE

The LDL Direct Cholesterol assay is a homogeneous method for directly measuring LDL-C levels in serum or plasma, without the need for any off-line pretreatment or centrifugation steps.

The method is in a two reagent format and depends on the properties of a unique detergent. This detergent (Reagent 1) solubilizes only the non LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non color forming reaction.

A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

REAGENT COMPOSITION

Reagent 1 : Enzyme Reagent
Reagent 2 : Developer Reagent
Calibrator : (Lyophilized) Human Serum.

SPECIMEN COLLECTION AND HANDLING.

Fresh serum is the recommended sample. EDTA or heparinized plasma are also acceptable.

Anticoagulants containing citrate should not be used. specimen.

Removed from the blood clot as soon as possible

Stability of the sample: 7 days at 2-8°C .

WORKING REAGENT PREPARATION.

Reagents are ready to use as supplied. **Reconstitute the calibrator with the exact volume of deionized water as mentioned on the label.** Mix well. Allow to stand at room temperature for 30 minutes.

REAGENT STORAGE AND STABILITY

When stored at 2-8°C reagent is stable until the expiration date stated on the bottle and kit box label.

REFERENCE VALUES

LDL (mg/dL)	
Optimal	<100
Near or above optimal	100-129
Borderline high	130-159
High	160-189

Very high		>190
AUTOMATED PARAMETERS		
Wavelength	546 nm	
Measurement	Against DI Water	
Cuvette	1 cm light path	
Reaction Temperature	37°C	
Reaction Type	End Point	
Reaction Direction	Increasing	
Incubation	5 Min. + 5 Min.	
Sample Volume	10 µL	
Reagent I Volume	750 µL	
Reagent II Volume	250 µL	
Low Normal	0 mg/dl	
High Normal	100 mg/dl	
Linearity	400 mg/dl	

MANUAL ASSAY PROCEDURE

PIPETTE INTO TEST TUBES

	CALIBRATOR	SAMPLE
REAGENT I	750 µl	750 µl
CALIBRATOR	10 µl	-
SAMPLE	-	10 µl
Mix well and incubate for 5 mins at 37°C & Immediately Add		
REAGENT II	250 µl	250 µl
After 5 minutes at 37°C. Read the absorbance (Ac) for calibrator and absorbance (As) for sample.		

CALCULATIONS:

Results are calculated, usually automatically by the instrument, as follows:

$$\text{LDL Chol (mg/dL)} = \frac{(\text{As}) \text{ of Unknown} \times \text{Calibrator value}}{(\text{Ac}) \text{ of Calibrator}}$$

NOTES:

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.

LINEARITY

The method is linear upto concentration of 400 mg/dl. Dilute samples above this concentration 1:1 and multiply the result by 2.

BIBLIOGRAPHY

Friedewald, W.T., Levy, R.I., and Fredrickson, D.S., Estimation of the low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge, Clin. Chem. 18, 1972, p. 449 - 502.